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Comparison of the Effects of High versus Low-Polyphenol Dark Chocolate on Body Weight and Biochemical Markers: A Randomized Trial

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Abstract

Background: Dark chocolate (DC) has amongst the highest content of polyphenols in foods, but the chocolate processing methods may greatly reduce this amount. Few studies addressed the possible detrimental effects of low polyphenol DC on body weight, glucose metabolism and lipid levels, and the potential role of cocoa flavanols in body weight control. Therefore, this study aimed to determine the effect of DC rich and DC low in polyphenols on BMI, fasting blood glucose, high sensitivity C-reactive protein (hs-CRP) and lipid levels in adults.

Methods: Sixty-one participants took part in a randomized parallel trial. Volunteers randomly received 20g daily of either PRDC (polyphenol-rich DC) or of low polyphenol DC (LPDC) for four weeks. Anthropometric measures and blood samples were collected at baseline and after 4 weeks.

Results: A significant net increase in BMI (0.17 ± 0.32 kg/m^2, p = 0.007), fasting blood glucose (0.44 ± 1.08 mmol/l, p = 0.041) and triglycerides levels (0.13 ± 0.23 mmol/l, p = 0.008) was observed in the low polyphenol DC group following the 4 weeks intervention, while the levels of these parameters did not significantly change in the polyphenol-rich DC group. There was no significant change in hs-CRP levels in both groups.

Conclusions: Results show that the intake of PRDC seems to be more metabolically healthy than LPDC intake, and this highlights the potential role of polyphenols in counteracting the negative effects of fat and energy intake in chocolate. The outcomes raise concerns about the polyphenol content and quality of DC products in the market. Further studies are needed to fully investigate the health benefits of dark chocolate intake, compare the effects of different types of chocolate and establish the necessary guidelines of the type and content of polyphenols in the chocolate preparations to ensure their favourable effect on health.

This trial was registered at clinicaltrials.gov as NCT01749020

Keywords: *Polyphenol-rich dark chocolate; BMI; Triglycerides; Low polyphenol dark chocolate; Glucose*

Background

Polyphenols constitute a class of phytochemicals widely available in plants and are largely abundant in the human diet [1]. Polyphenols have been previously implicated in many health benefits including prevention and management of diabetes, cardiovascular diseases (CVD), cancer and neurodegenerative diseases. These effects may be explained by the antioxidant capacity of polyphenols [2,3]. Recently, polyphenols have been involved in the modification of physiological and molecular mechanisms related to adiposity and energy metabolism [4] suggesting a role for these components in obesity and body weight control. As obesity (particularly visceral obesity) may lead

to low-grade inflammatory state that causes insulin resistance and endothelial dysfunction [5], lowering inflammation might improve glucose metabolism by increasing insulin sensitivity.

Dark chocolate consists of cocoa liquor and cocoa butter (contains 33% of stearic acid, 33% of oleic acid and 25% of palmitic acid) [6], and is one of the major contributors to polyphenol intake in the western diet [7]. Cocoa polyphenols mainly consist of flavanols (including epicatechin, catechin and procyanidins) and quercetin-type flavanols [8]. Flavanols, along with theobromine and caffeine, are responsible for the astringent taste of chocolate [9,10]. Nonetheless, the amount of polyphenols in DC mainly depends on the method of processing. Examples of such methods are fermentation and dutching, which result in improved flavour and taste but might cause a significant loss or damage of polyphenols [10]. Hence, the amount of flavanols in two different commercial types of chocolate with equal cocoa concentrations can markedly differ from one chocolate to another, depending on the level of processing [10,11]. In the last decade, many studies highlighted the effects of cocoa/chocolate on health due to its high polyphenol/flavanols content [12]. However, few other studies discussed the potential detrimental effects of a low polyphenol chocolate on biochemical and anthropometric markers [13-15], and the possible role of polyphenols in countering the negative effects of fat (from cocoa butter) in chocolate [13,16]. Indeed, DC can be a significant source of fat and sugar [10]. Animal studies have been promising in demonstrating that polyphenols (and specifically flavanols) in cocoa decrease body weight gain in rats and mice [17-19]. In addition, a human observational study including 1018 individuals showed an inverse correlation between high chocolate consumption and BMI [20]. Overall, with DC products being increasingly marketed for their health benefits, and knowing that processing methods may cause a substantial decrease in the DC polyphenol content, it is important to explore whether benefits DC consumption apply to all types of dark chocolate in the market; or alternatively, that some DC products deficient in polyphenols might not produce such favourable effects, and thus be detrimental to health. Therefore, this study aimed to elucidate the beneficial effects of PRDC on biochemical (including glucose and serum lipid levels) and anthropometric (BMI, waist circumference) measurements, and to identify any potential adverse effects resulting from LPDC consumption.

Subjects and Methods

The ethical approval for the study was granted by the division of Health Sciences Ethics Committee at Queen Margaret University (QMU). The studies were performed according to the declaration of Helsinki [21]. Participants were provided a written information sheet, and were asked to sign a consent form before taking part.

Study Design

The study was a randomized single-blinded controlled parallel study where participants received either 20g of LPDC or PRDC daily for a period of 4 weeks. A summary of the nutritional and chemical composition of the LPDC and the PRDC is presented in Table 1. The two types of chocolate looked identical, and contained 63.5% of cocoa solids. In order to avoid confounding factors, the two dark chocolate types were matched for macronutrient and micronutrient content, and contained similar amounts of caffeine and theobromine. They only differed in polyphenols content; with the PRDC providing 500 mg total polyphenols (400 mg flavanols) and the LPDC substantially less (< 60 mg flavanols). The products were provided by Barry-Callebaut Company, Belgium. PRDC and LPDC were produced via a method called "Acticoa" - patented trademark of Barry-Callebaut, which aims to naturally preserve polyphenols throughout the different steps of the manufacturing chain [22].

Participants were asked to attend two appointments (at baseline and after 4 weeks) which took place at Queen Margaret University research lab. BMI and waist circumference (WC), fasting blood glucose, hs-CRP and lipid levels (secondary outcomes) were assessed at each appointment. Randomization was implemented using sealed envelopes based on the protocol described by Doig and Simpson [23]. Standard-sized sheets were marked by either L (LPDC) or R (PRDC). The sheets were inserted into unlabelled envelopes and sealed. Then, the researcher or a member of the research team chose an envelope for each participant during the first appointment.

Prior to the appointment, volunteers were asked to be in a fasting state, to avoid drinking alcohol for the past 24 hours, and to consume the last dose of DC at least 12 hours before the second appointment. In addition, they were requested to refrain from doing a high

level of physical activity at least 12 hours prior to the visit, and to avoid or reduce eating high quantities of foods rich in flavonoids (> 4 mg/100g of selected foods [24]) one week before the start (run-in phase) and subsequently for the duration of the study. A list of foods rich in flavonoids (such as bananas, all berries, green tea and coffee) was provided to participants. Instructions were given before assigning participants to a particular group.

Components (per 20g daily portion)	LPDC	PRDC
Energy (kcal)	102	102
Total fat (g)	7.34	7.34
Carbohydrates (g)	7.44	7.44
Protein (g)	1.34	1.34
Total flavanols (mg)	< 60	400
Epicatechin (mg)	12	85
Catechin (mg)	2	15
Caffeine (mg)	15	15
Theobromine (mg)	150	150

Table 1: *Nutritional and chemical composition of the experimental dark chocolate.*
Data obtained from Barry-Callebaut company, Belgium. Total flavanols, epicatechin and catechin were analysed via LCMS method (Liquid chromatography–mass spectrometry). The amount of total polyphenols in the PRDC (500 mg) was assessed by Folin-Ciocalteu method, as provided by the company. The amount of 20g was the daily portion given to participants in the trial. The amounts of polyphenols, caffeine and theobromine provided by the company were validated by studies at the James Hutton Institute, Dundee, UK. PRDC: Polyphenol-rich dark chocolate; LPDC: Low polyphenol dark chocolate

Study population

Volunteers were recruited via University email, flyers and posters in community and sports centres, hospitals, colleges and universities in Edinburgh, as well as via word-of-mouth. Participants were studied with no restriction to race or socioeconomic status according to the following criteria 1) Adults with no history of hypertension, type 2 diabetes or CVD; 2) Participants with BMI between "18.5-24.9 kg/m^2" and BMI between "25-34.9 kg/m^2"; 3) Males and Females and 4) Age: 18-65 years. Exclusion criteria included history of CVD, hypertension, diabetes, intake of medications that affect insulin, glucose, lipids, and/or hs-CRP levels, intake of dietary supplements containing high doses of antioxidants (only low amounts of multivitamins were considered acceptable doses), and current smoking and heavy alcohol drinking (defined as more than 15 drinks/week for men and more than 8 drinks for women [25]). In addition, postmenopausal women receiving hormone replacement therapy, and participants with regular consumption of cocoa or DC (> 1 serving/week) [7] were not eligible for participation.

Blood sampling and anthropometric measures

Blood samples and anthropometric measurements (weight, height, and waist circumference) were taken before the start and at the end of the intervention (after 4 weeks). Height was measured twice with person barefoot using a stadiometer to the nearest 0.5 cm. Weight was measured in the morning at fasting using an electronic scale (Tanita BF-559, Body Fat Monitor/Scale) to the nearest 0.1 Kg. Waist circumference was measured via a metal measuring tape calibrated against a steel tape, and was placed around the waist at the middle point between the lowest rib and the top of the hip bone. The tape was snug (without compressing the skin) and parallel to the floor [26]. Samples of blood were collected from the antecubital vein with minimal stasis by a butterfly needle (Vacuette®, Greiner bio-one). The blood sample drawn at each time was around or less than 20 ml. The samples were then centrifuged for 10 min at 4°C and 3000 rpm (revolutions per minute; Thermo Scientific Heraeus Primo R centrifuge). The supernatant plasma was then harvested, and stored frozen in polystyrene tubes at -80°C for subsequent analysis. Fasting serum lipids (Total cholesterol = TC, Triglyceride = TG, LDL

and HDL), glucose and hs-CRP levels were measured. The analysis of glucose and lipids profile in plasma samples was undertaken at the Queen's Medical Research Institute, University of Edinburgh using an automated platform (COBAS, UK). The technician was blinded to randomisation assignment. Hs-CRP levels were analysed at QMU lab via high sensitivity enzyme immunoassay (ELISA method) (Genway Biotech Inc, USA). Hs-CRP was considered as the test of choice when assessing inflammation in individuals with no history of CVD [27].

Questionnaires and diet diaries collection

Subjects were requested to complete a general questionnaire covering information on social characteristics, lifestyle habits and medical history. Participants were asked to maintain their life style throughout the intervention period, while avoiding flavanol-rich foods and substituting the experimental DC by another food. A diet adjustment was also considered so that DC possibly replaced another food of similar energy. The researcher helped in providing dietary advice for participants to help them achieve this goal. This happened by asking participants about the types of snacks usually consumed. Energy composition of these snacks was assessed, and substitutions based on food energy content were suggested by the researcher. To monitor changes in dietary intake, participants were asked to fill a 3-day un-weighted diet diary twice: at the run-in phase and at week 3. Energy and macronutrient intakes were then analysed using NetWISP dietary software package (V 3.0). Changes in physical activity levels were assessed via a physical activity questionnaire at the run-in phase and during the second appointment (at week 4). Physical activity levels were then transformed into MET (Metabolic equivalent task)/hour. Furthermore, an acceptability questionnaire was designed to obtain data on whether it was palatable/acceptable to consume the assigned portion of dark chocolate daily (either LPDC or PRDC). Compliance to the study protocol was assessed by: 1) through the 3-day diet diary and physical activity questionnaire and 2) Directly asking participants whether they have consumed all the samples of chocolate daily as required. A high compliance was defined as the consumption of 85% or more (equivalent to missing no more than one sample a week) of the chocolate throughout the study.

Statistical analyses

Continuous normally distributed data were expressed as mean ± SD. Data were analysed using SPSS for Windows version 19.0 (SPSS, Chicago, IL). Heterogeneity was assessed using Levene's test for equality of variances. Differences in baseline characteristics between groups were examined using a two-tailed independent t-test. For within group comparisons, changes from baseline were analysed using a two-tailed student's paired t-test. For between-group differences, ANCOVA was carried out to adjust for potential baseline differences in the outcomes. Non parametric data were analysed using Mann Whitney tests. Significant changes were set at $p \leq 0.05$.

Results

The number of volunteers who completed the trial was 61 out of 64 subjects recruited. Two participants in the LPDC group (week 2 and week 3) and one in the PRDC group (week 2) dropped out during the intervention. Reasons for dropouts were infection, travel commitments, and one participant decided to drop out after starting a migraine medication. According to the BMI, 39 participants had a BMI < 25 Kg/m^2, and 22 participants had a BMI > 25 Kg/m^2. The baseline characteristics of the study population are presented in Table 2.

	Study Population (n = 61)	LPDC (n = 30)	PRDC (n = 31)	Difference Between Groups P
Age (years)	28.82 ± 8.89	28.13 ± 8.98	29.48 ± 8.89	0.48
Gender (M/F)	12/ 49	3/27	9/22	0.062
BMI (Kg/m^2)	23.92 ± 4.17	24.08 ± 3.78	23.77 ± 4.57	0.4
WC (cm)	77.7 ± 10.52	76.83 ± 8.89	78.54 ± 11.98	0.14

Table 2: *Baseline characteristics of study participants. There were no significant differences between the LPDC and the PRDC groups for age, gender, weight, BMI and WC when assessed via two-tailed independent t-test (p > 0.05). Mean ± SD for all such values. LPDC: Low polyphenol-dark chocolate; PRDC: Polyphenol-rich dark chocolate; WC: waist circumference.*

Biochemical assessment

There was no significant change in blood glucose levels in the PRDC group after 4 weeks. However, glucose levels increased in the LPDC group (Figure 1). In addition, TC, HDL and LDL levels did not significantly change throughout the intervention in both groups ($p > 0.05$), but TG levels increased significantly in the LPDC group but not significantly in the PRDC group (Table 3). No significant changes in the inflammatory marker hs-CRP in both PRDC group (from 1.35 ± 0.97 mg/L at baseline to 1.26 ± 0.88 mg/L after 4 weeks; Δ = -0.09 ± 0.2 mg/l, $p = 0.32$), and LPDC group (from 1.5 ± 1.04 mg/L at baseline to 1.54 ± 1.12 after 4 weeks; Δ = 0.04 ± 0.8 mg/L, $p = 0.57$) were noted.BMI status did not have a significant impact on the outcomes: there was no difference in the response to treatment when participants were stratified according to BMI (BMI < 25 Kg/m^2 or BMI > 25 Kg/m^2) in the LPDC (for TC, $p = 0.35$; LDL, $p = 0.7$; HDL, p = 0.25; TG, $p = 0.59$) and hs-CRP ($p = 0.56$), and the PRDC (for TC, $p = 0.73$; LDL, p = 0.44; HDL, $p = 0.58$; TG, $p = 0.13$) and hs-CRP ($p = 0.35$) groups.

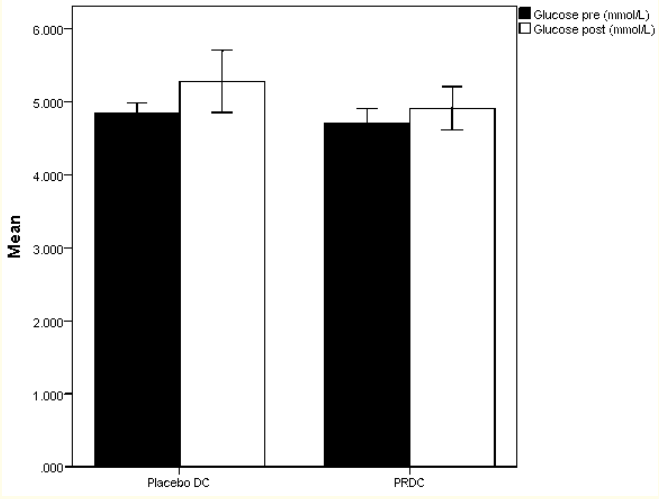

Figure 1: *Changes in glucose levels in the LPDC and PRDC after 4 weeks.*
LPDC: Low polyphenol-dark chocolate, PRDC: Polyphenol-rich dark chocolate.
** Significant difference from pre intervention (baseline), p = 0.041). Data were analysed using paired-t-test. Values expressed as means mean ± SEM.*
There was no significant difference in baseline glucose levels between the LPDC and PRDC groups (p = 0.27), when assessed via independent t-test.

Lipid levels (mmol /L)	Pre-PRDC Mean ± SD	Post-PRDC Mean ± SD	Δ (Post-PRDC - Pre-PRDC)	p	Pre- LPDC Mean ± SD	Post-LPDC Mean ± SD	Δ (Post-LPDC - Pre- LPDC)	p
TC	4.17 ± 1.07	4.23 ± 0.98	0.059 ± 0.76	0.68	4.26 ± 0.79	4.5 ± 1.15	0.24 ± 0.91	0.18
LDL	2.23 ± 0.97	2.22 ± 1.06	-0.013 ± 0.66	0.92	2.23 ± 0.8	2.35 ± 1.08	0.12 ± 0.73	0.43
HDL	1.56 ± 0.36	1.64 ± 0.34	0.08 ± 0.27	0.12	1.66 ± 0.35	1.73 ± 0.4	0.07 ± 0.24	0.12
TG	0.83 ± .0.46	0.69 ± 0.23	-0.14 ± 0.43	0.07	0.83 ± 0.32	0.96 ± 0.37	0.13 ± 0.23*	0.008

Table 3: *Changes in serum lipid levels from baseline following the intervention.*
**Significant difference from pre intervention, p < 0.05. Data analysed using paired-test. Mean ± SD for all values*
TG: Triglycerides; WC: waist circumference; LPDC: Low polyphenol-dark chocolate; PRDC: Polyphenol-rich dark chocolate.

Anthropometric measures

Analysis of differences from baseline showed that weight, BMI and WC did not significantly change in the PRDC group, whereas four weeks of daily DC low in polyphenols consumption increased weight in the LPDC group (Table 4). Subgroup analysis based on BMI (BMI < 25 Kg/m^2 or BMI > 25 Kg/m^2) did not show a difference in gained weight between normal weight and overweight participants in the LPDC group, when assessed via Mann Whitney test (p = 0.36).In the LPDC group, the mean difference (post-pre) (IQR) was 0.12 Kg/m^2 (0.41) for the normal weight population and 0.24 Kg/m^2 (0.48) for the overweight population.

	Pre-PRDC Mean ± SD	Post-PRDC Mean ± SD	Δ (Post-PRDC - Pre-PRDC)	P	Pre-LPDC Mean ± SD	Post-LPDC Mean ± SD	Δ (Post-LPDC - Pre-LPDC)	P
BMI (Kg/m^2)	23.77 ± 4.57	23.76 ± 4.65	-0.01 ± 0.3	0.82	24.08 ± 3.78	24.25 ± 3.87	0.17 ± 0.32	0.007*
WC (cm)	78.54 ± 11.98	78.75 ± 11.8	0.21 ± 1.7	0.5	76.83 ± 8.89	76.96 ± 8.98	0.13 ± 0.84	0.42

Table 4: *Changes in anthropometric measurements in LPDC and PRDC groups following the intervention.*

** Significant difference from pre intervention, p < 0.05. Data analysed using paired-test. Mean ± SD for all values.*

LPDC: Low polyphenol-dark chocolate; PRDC: Polyphenol-rich dark chocolate.

ANCOVA measures were performed for the above assessed parameters, in order to assess any impact of baseline imbalances on the results. Analysis showed significant differences between the LPDC and PRDC groups for TG (p < 0.001), BMI (p = 0.028) and weight (p = 0.031), but not for waist circumference (p = 0.76), glucose (p = 0.27), hs-CRP (p = 0.31), TC (p = 0.35), HDL (p = 0.82) and LDL (p = 0.51). None of the above mentioned variables violated the assumption of homogeneity of variance (Levene's test > 0.05).

Assessment of diet diaries and compliance

Compliance was estimated to be 87%. This was calculated by asking participants about the number of times they have missed the daily portion of chocolate over the study period (28 days). The percentage of compliance was calculated for each participant, and the average percentage was considered for the 61 participants. Reported physical activity levels converted into metabolic equivalent task (MET) per week did not significantly change throughout the intervention in the LPDC group (Pre: 14.35 ± 14.01 MET/week; post: 13.32 ± 14.76 MET/week, p = 0.36) and the PRDC group (Pre: 21.08 ± 17.09 MET/week; post: 20.37 ± 16.78 MET/week, p = 0.54). All participants provided self-reported diet diaries. Paired-sample analysis on the NetWISP software (V3.0) showed no significant differences in energy and macronutrient intake following the intervention in both groups. Participants whether on either the LPDC or PRDC have maintained their usual calorie intake throughout the study (Table 5).

		Run-in period	Week 3	Significance (p=)
Energy (Kcal)	LPDC (N = 30)	1799 ± 522	1746 ± 416	0.54
	PRDC (N = 31)	1868 ± 564	1998 ± 644	0.1
Carbohydrate (g)	LPDC (N = 30)	226.9 ± 64	208.3 ± 58	0.28
	PRDC (N = 31)	233 ± 61	234 ± 62	0.93
Protein (g)	LPDC (N = 30)	64 ± 22.2	63.2 ± 19.5	0.99
	PRDC (N = 31)	73 ± 41.7	84 ± 49.5	0.07
Fat (g)	LPDC (N = 30)	73.2 ± 32	73.4 ± 25	0.96
	PRDC (N = 31)	72.6 ± 29.3	82.7 ± 34.3	0.08

Table 5: *Mean differences in energy and macronutrient intakes between the run-in period and week 3.*

Differences between run-in period and week 3 were not significant for energy and macronutrient, p > 0.05.

Data expressed as mean ± SD. Results were analysed using paired tests on the NetWISP dietary software package (V3).

LPDC: Low polyphenol-dark chocolate; PRDC: Polyphenol-rich dark chocolate.

Acceptability of treatment

With regard to acceptability, it was found that LPDC was more acceptable to consume than PRDC (Figure 2). The reasons given for unacceptability were mainly the bitterness and intense flavour, the texture, and few subjects reported some side effects: nausea, bloating, winds, stomach pain, and to a less extent the dark colour of the chocolate which made it less appealing. These complaints, although noteworthy, did not result in any dropout.

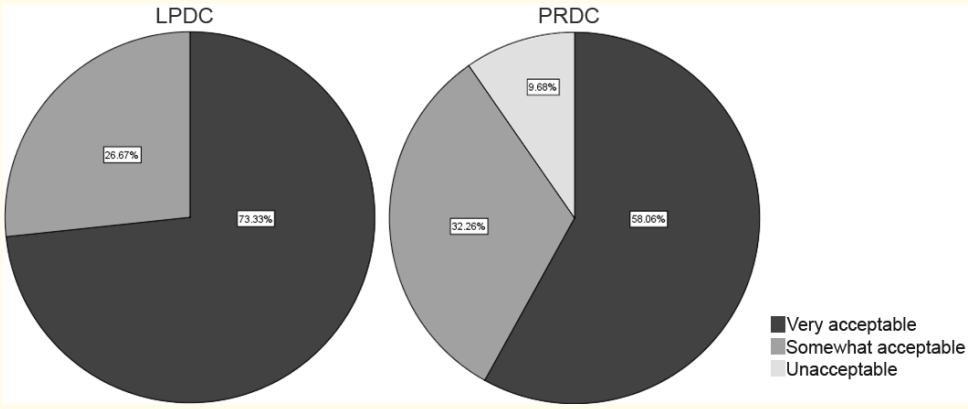

Figure 2: *Acceptability of treatment based on the type of intervention.*

LPDC: Low polyphenol dark chocolate, PRDC: Polyphenol-rich dark chocolate.

Discussion

Findings of this study showed that DC low in polyphenols increased BMI, glucose and TG levels in the LPDC group, whereas none of these parameters significantly changed in the PRDC group. However, no blank group was carried out in this study (see limitations section). These parameters were recognized as being risk factors for chronic diseases [28]. The increase in weight (0.57 ± 1.08 kg, $p = 0.011$) following 4 weeks of LPDC consumption was only reported by the study of Al-Moosawi., *et al*. [13] in which the LPDC composition was similar to this study. In relation to lipid profile, a detrimental effect of low-polyphenol chocolate was reported by the study of Wang-polagruto., *et al*. [15], which showed that consuming a low flavanol cocoa drink (43 mg of flavanols) resulted in a decrease in HDL by 9.6%, while an increase in HDL by 6.6 % was noted in the high flavanol cocoa drink (446 mg of flavanols). Also, Baba., *et al*. [29] reported a beneficial effect of cocoa polyphenols on LDL oxidation and evidenced by a 9.4% increase in lag time for LDL oxidation after 12 weeks of flavanol-rich cocoa consumption ($p < 0.001$). However, a significant decrease (19.8%) in the lag time for LDL oxidation was noted in participants in the control group (consuming 12g of sugar) [29]. Regarding the inflammatory marker hs-CRP, our findings match those obtained by most of the intervention studies on dark chocolate which showed no significant effects of LPDC or PRDC on hs-CRP levels [30-33]. Hence, despite the increase in weight, low-polyphenol chocolate did not result in a significant change in this inflammatory marker. This could be due to the short term intervention of this study.

The increase in BMI, glucose and TG levels only in the LPDC group but not in the PRDC group, might suggest a possible counteracting effect of PRDC (500 mg of polyphenols) on the adverse effects of the fat content of chocolate. In line with this, it was proposed that cocoa polyphenols might counteract the pro-oxidant effects of cocoa butter, and thus could help against an increase in weight as well an increase in glucose levels [16]. The outcomes could possibly be in agreement with the findings of animal studies which showed that cocoa polyphenols reduced weight gain in animals when administered high fat diets [17,19,34]. Although the aim of our study was to include the LPDC and PRDC in the context of a normocaloric diet, the increase in BMI might suggest that this product was consumed in addition to the diet of participants. Nevertheless, the analysis of diet diaries did not show a significant increase in energy intake between baseline and week 3 in LPDC and the PRDC groups. This might be mostly caused by under-reporting, which is common in

research studies [34]. Indeed, under-reporting, along with overestimating physical activity, was previously documented in a cocoa study [35]. Therefore, we could suggest that participants of this study did not adhere to the dietary recommendations provided, and consumed the chocolate portion in addition to their diet, instead of substituting it for another food. Yet, the polyphenol content in the PRDC might have counteracted the harmful effects of increased energy and fat in DC, and prevented an increase in weight in the PRDC group.

The mechanism involved in the countering effect of PRDC on weight gain might be explained by the inhibitory effect of PRDC on fatty acid gene expression [17] or on digestive enzymes [37]. The implication of the latter mechanism might provide an explanation for the adverse effects (such as bloating) noted in some subjects of this study. In fact, this might be due to changes in bowel function caused by the fractions of fat and carbohydrate that were unabsorbed. As the possible mechanisms were mainly studied in animals and *in vitro* cell studies, human studies investigating the mode of action of polyphenols would then be required.

Our results suggest that common chocolate products in the market (assumed to be of unknown or low in active polyphenols contents) could cause unfavourable effects on health. In addition, the outcome on TG levels refutes the theory that chocolate consumption has presumably a neutral effect on lipid profile [12]. Our findings raise concerns over the different types of DC in the market, as certain current methods of processing might cause a considerable loss or damage of polyphenols [38]. In fact, chocolate companies may choose to increase product palatability by masking the astringent flavour of flavanols and thereby increasing consumer acceptability. This may involve the use of processing methods that might cause major polyphenol damage or losses. Indeed, qualitative data from our study reinforces the idea that low polyphenol DC is more acceptable and preferable than high polyphenol DC due to its reduced bitterness taste. If evidence for the health benefits of DC continues to accrue, studies that confirm the content of polyphenols in DC in the market may become crucial, and indeed the need for legislation regarding the labelling of polyphenol content of chocolate could be considered.

It is important to mention that although changes in the LPDC group (increase in BMI and Triglycerides levels) were small and might not be considered clinically relevant, a longer term study might have led to larger and more relevant changes. Therefore, results obtained constitute an important basis for carrying out larger studies investigating the long term effects of PRDC and LPDC on anthropometric, biochemical and physiological markers.

Limitations

Some limitations are inherent to this study. The short study duration did not elucidate whether the effects noted are purely short term or might persist for a longer term. Women represented the largest proportion of both arms of the study, which may limit the generalization of the results. Furthermore, an inability to control the diet of participants resulted from the inclusion of free-living individuals who commonly exhibit a large variability in their dietary habits [39]. Therefore, we cannot exclude the possibility that they consumed other polyphenol-rich foods. Moreover, the compliance to intervention was not assessed by analysing total polyphenols in a 24-hour urine samples as a biochemical marker for compliance. Despite the limitations related to this test, it might have provided a better indication of adherence [40]. Also, body fat percentage was not assessed to indicate whether the increase in weight was caused by an increase in fat mass. In addition, differences in abiding to the dietary instructions provided by the researcher prior to the study can constitute an important limitation. The results of this study may not apply to regular DC consumers, who were among the exclusion criteria; and due to the small changes observed, it cannot be ruled out that the inter individual differences in the bioavailability of polyphenols might have affected the results. Importantly, including a third arm in this study (blank control with no experimental chocolate) would have been helpful in providing a more valid explanation for the results obtained. Lastly, the effects obtained in the LPDC group might be attributed to the restriction of polyphenols intake in the LPDC group (as instructed) that might caused a change in their usual diet. Therefore, these results constitute a preliminary finding and justify carrying out further studies to elucidate the effect of low polyphenol DC and other chocolate products on anthropometric and biochemical markers.

Conclusion and Directions for Future Research

Despite the fact that our study showed very small changes in BMI and blood glucose following the intake of low polyphenol DC, it highlights the issue that not all DC brands in the market may have health benefits. Thus, the analysis of the polyphenol content in the chocolate products consumed by the general population might be necessary to elucidate any differences. This could suggest a move towards stating the amount of polyphenols as a part of a labelling requirement, and efforts in relation to unifying or standardising the processing methods of cocoa could also be considered. Standardising polyphenol levels would be a first step to the inclusion of dark chocolate products in any future public health recommendations. Polyphenols might be beneficial in counteracting the negative effects of the high fat and energy in chocolate and other nutrients. Finally, because of the lower acceptability of PRDC compared to DC low in polyphenols, increasing the tolerability of PRDC should probably be considered, and further research investigating ways of improving the taste of chocolate rich in polyphenol is justified.

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Volume 2 Issue 3 September 2015